

Spot variation FCS allows for superresolution chronoscopy of confinement times in membranes

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Supplementary Notes

Note 1: Time-resolution in Standard FCS

If timings are appropriate, conventional FCS can be used to measure trapping times of a diffusing tracer molecule (1, 2). Michelman-Ribeiro et al. provided in-depth estimations of the achievable time-resolution in 3-dimensional FCS (1). In Figure 1 of their paper they distinguished specific regimes depending on the kinetic parameters $k_{on}^* = 1/\tau_{free}$ and $k_{off} = 1/\tau_{trap}$. Only the regime with rare trapping events in the focal spot $n = \tau_D'/\tau_{free} \ll 1$ provides any access to kinetic parameters, all other regimes show effective diffusion. The region specified by $n \ll 1$ was further characterized. The reaction-dominant case around $\tau_{trap}/\tau_{free} \approx 0.5$ allows for measuring all parameters of the model (on- and off-rates and the diffusion constant). In this case, the two-dimensional ACF is given by (1, 3)

$$G(t) = \frac{1}{N} \left[(1 - \beta) \frac{1}{1 + \frac{t}{\tau_D'}} + \beta \exp\left(-\frac{t}{\tau_{trap}}\right) \right]$$

From the pronounced shoulders, the two characteristic time scales τ_D' and τ_{trap} and the proportion $\beta = \tau_{trap}/(\tau_{trap} + \tau_{free})$ can be easily extracted. A hybrid case with high values of β , and the full model case were further discriminated, both providing access to the trapping time τ_{trap} .

Note 2: Stop-and-Go Diffusion with Non-Exponential Trapping Times

For the derivation of Eq. 3 we assumed that the penalty due to the first trapping event is identical to the mean trapping time τ_{trap} , which is justified by the choice of an exponential trapping time distribution. However, if we assume distributions with memory effects we generally find penalties deviating from the mean. In the following, we provide formulas for Gamma-distributed and constant trapping times (both short-tailed distributions), and for the Weibull distribution (long-tailed for shape-factor $k < 1$) (Fig. 3).

For a general trapping time distribution $p(t_{trap})$, the average time penalty t_1 is given by

$$\langle t_1 \rangle = \int_0^\infty \int_0^{t_{trap}} t_1 p_2(t_1 | t_{trap}) p_1(t_{trap}) dt_1 dt_{trap}$$

with

$$p_1(t_{trap}) dt_{trap} = \frac{t_{trap} p(t_{trap}) dt_{trap}}{\int t_{trap} p(t_{trap}) dt_{trap}}$$

denoting the probability to select for a trapping interval of length t_{trap} , and

$$p_2(t_1 | t_{trap}) dt_1 = \frac{1}{t_{trap}} dt_1$$

the conditional probability to obtain a penalty t_1 given a trapping time t_{trap} (Suppl. Fig. S1). We thus obtain:

$$\langle t_1 \rangle = \frac{1}{2\tau_{trap}} \int_0^\infty t_{trap}^2 p(t_{trap}) dt_{trap} = \frac{\langle t_{trap}^2 \rangle}{2\tau_{trap}}$$

or more generally

$$\langle t_1 \rangle = \frac{1}{2} \left(\frac{\text{Var}(t_{\text{trap}})}{\text{Mean}(t_{\text{trap}})} + \text{Mean}(t_{\text{trap}}) \right)$$

$\langle t_1 \rangle$ can therefore deviate from the mean and may take any value above $\tau_{\text{trap}}/2$, depending on the distribution. The generalization of **Eq. 3** then reads

$$\tau_0 = \beta \langle t_1 \rangle = \beta \tau_{\text{trap}} f_{\text{distr,trap}}$$

In particular, the conditions $\langle t_1 \rangle \gtrless \tau_{\text{trap}}$ are equivalent to $\text{Std}(t_{\text{trap}}) \gtrless \text{Mean}(t_{\text{trap}})$. In other words, a distribution yields an offset larger (smaller) than an exponential distribution if its standard deviation is larger (smaller) than its mean.

In the following, we provide calculations for various distributions:

- i) **Exponentially-distributed** trapping time with mean τ_{trap}

$$f_{\text{distr,trap}} = 1 \quad \text{Eq. S1}$$

- ii) **Constant** trapping time τ_{trap} :

$$f_{\text{distr,trap}} = 1/2 \quad \text{Eq. S2}$$

- iii) **Gamma-distributed** trapping time with mean τ_{trap} and shape-factor k :

$$f_{\text{distr,trap}} = \left(\frac{1}{2} + \frac{1}{2k} \right) \quad \text{Eq. S3}$$

For $k \rightarrow \infty$ a normal distribution with zero variance is approached, approximating $f_{\text{distr,trap}} \rightarrow 1/2$. $k = 1$ recovers the exponential distribution with $f_{\text{distr,trap}} = 1$.

- iv) **Weibull-distributed** trapping time with mean τ_{trap} and shape-factor k :

$$f_{\text{distr,trap}} = \frac{1}{2} \left(\frac{1}{\Gamma(1+\frac{1}{k})^2} \Gamma\left(1 + \frac{2}{k}\right) \right) \quad \text{Eq. S4}$$

Again, for $k \rightarrow \infty$ a normal distribution with zero variance is approached, approximating $f_{\text{distr,trap}} \rightarrow 1/2$; $k = 1$ recovers the exponential distribution with $f_{\text{distr,trap}} = 1$. For $k \rightarrow 0$ $f_{\text{distr,trap}}$ diverges.

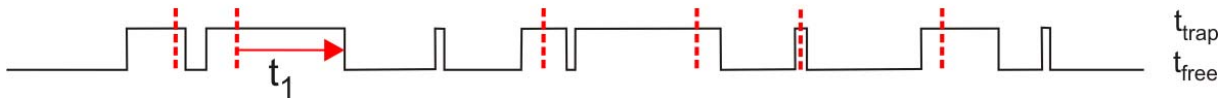


Figure S1. Transition sequence between free diffusion (lower level) and trapped state (upper level). The time penalty t_1 specifies the average time a tracer remains in a particular trapped state. Red dashed lines indicate potential events for catching a molecule in a trapped state.

Note 3: Stop-and-Go Diffusion with Non-Exponential Free Diffusion Times

The derivation of Eq. 3 was based on the assumption that the diffusion process has no memory; in particular, release from the trap is followed by an average diffusion time τ_{free} . However, if the diffusion time follows a non-exponential distribution $p_{free}(t_{free})dt_{free}$, the subsequent number of stops n within the given time interval will change. For estimating the transit time τ_D let us therefore focus now on the trapped fraction β , and calculate the number of stops within τ_D' . The situation is illustrated in the timeline shown in **Suppl. Fig. S2**. At an arbitrary position within the timeline the average number of stops is given by $n = \tau_D'/\tau_{free}$. By shifting the time interval depicted in red to the nearest neighboring spot on the left side, n will in general be altered to n' .

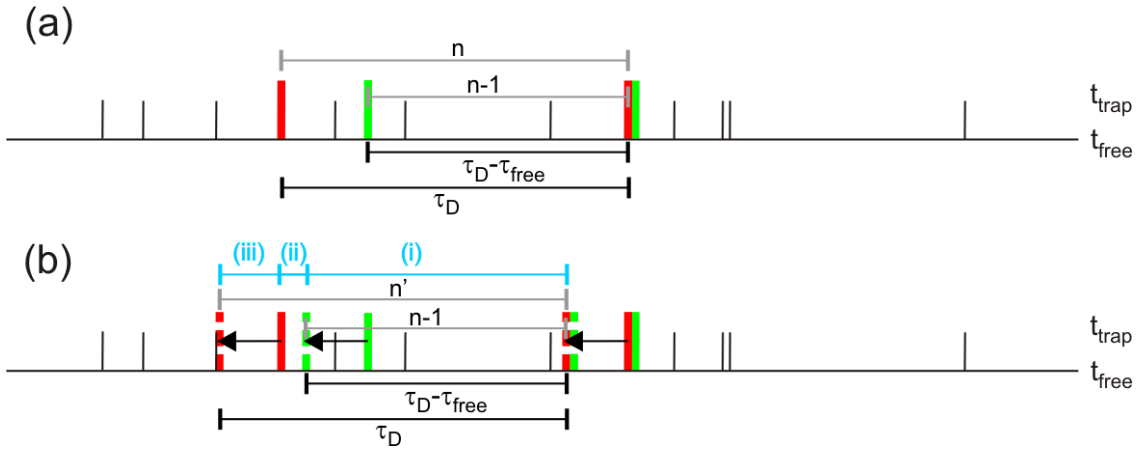


Figure S2. Time sequence of trapping events (vertical lines) separating periods of free diffusion. (a) shows an arbitrary time interval τ_D' (red lines), during which the tracer is supposed to transit the focal area. During this interval, the tracer experiences an average of n stops. We further introduce a second interval of length $\tau_D' - \tau_{free}$, which is bounded by the same right border and a new left border (green line); this interval contains on average $n-1$ stops. (b) We shift the red interval to the nearest trapped state at the left (red dashed lines); the linked green interval follows accordingly. For estimating the number of trapped states in the new red interval n' , we sum up the individual contributions (from right to left): $n-1$ stops originating from the green interval (i) and $\langle t_1 \rangle / \tau_{free}$ stops originating from the time period between the former red left border and the new green left border (ii); the time period (iii) does not contain further stops.

For calculating n' , let us revert to a new time interval of length $\tau_D' - \tau_{free}$, which contains on average $n-1$ traps (green in **Suppl. Fig. S2**). The green interval shall contain the same right border as the red interval. By shifting the two intervals to the nearest left neighbor, the new green interval still contains $n-1$ traps (for this we assumed that two successive intervals are not correlated). No additional trap is added due to shifting the left border of the red interval. The probability that one trap is contained in the remaining interval between the left borders of the old red interval and the new green interval is given by $\langle t_1 \rangle / \tau_{free}$, where $\langle t_1 \rangle$ denotes again the average time from any arbitrarily chosen position to the next trap. $\langle t_1 \rangle$ is specified by Eqs. S1-S4 upon replacing τ_{trap} by τ_{free} .

Together, we find an average of $n' = \langle t_1 \rangle / \tau_{free} + n - 1$ stops. The total transit time is thus given by

$$\begin{aligned} \tau_D &= \beta \left[\tau_D' + \left(\frac{\langle t_1 \rangle}{\tau_{free}} + n - 1 \right) \tau_{trap} + \tau_{trap} f_{distr_1, trap} \right] + (1 - \beta) \tau_D' + (1 - \beta) n \tau_{trap} \\ &= \frac{\omega^2}{4D_{free}(1 - \beta)} + \beta \tau_{trap} (f_{distr_2, free} + f_{distr_1, trap}) \end{aligned}$$

with $f_{distr_2,free} = \frac{\langle t_1 \rangle}{\tau_{free}} - 1$. Using Eqs. S1-S4, we obtain the following contributions to the offset (**Fig. 3**):

$$f_{exp,free} = 0 \quad \text{Eq. S5}$$

$$f_{constant,free} = -1/2 \quad \text{Eq. S6}$$

$$f_{gamma,free} = -\frac{1}{2} + \frac{1}{2k'} \quad \text{Eq. S7}$$

$$f_{weibul,free} = \frac{1}{2} \left(\frac{1}{\Gamma(1+\frac{1}{k})^2} \Gamma\left(1 + \frac{2}{k}\right) \right) - 1 \quad \text{Eq. S8}$$

References

1. Michelman-Ribeiro, A., D. Mazza, T. Rosales, T. J. Stasevich, H. Boukari, V. Rishi, C. Vinson, J. R. Knutson, and J. G. McNally. 2009. Direct measurement of association and dissociation rates of DNA binding in live cells by fluorescence correlation spectroscopy. *Biophys J* 97:337-346.
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